

Phytochemical, Antimicrobial and Antioxidant Evaluation of *Hibiscus rosa sinensis* Extract

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Abstract

The growing concern over antibiotic resistance has led to an increased interest in natural antibacterial agents. *Hibiscus rosa-sinensis*, a widely known medicinal plant, has demonstrated promising antibacterial properties due to its rich phytochemical profile. This study investigates the antibacterial efficacy of hibiscus flower extracts against *Salmonella typhi* and *Staphylococcus aureus*, two clinically significant pathogens. The extract was prepared using methanol and subjected to antimicrobial susceptibility testing (AST) using the Kirby-Bauer disk diffusion and agar well diffusion methods. Minimum Inhibitory Concentration (MIC) was determined to establish the lowest effective concentration. Phytochemical screening confirmed the presence of bioactive compounds such as tannins, flavonoids, coumarins, volatile oils, and saponins, which contribute to antimicrobial action. Results indicate that hibiscus extract effectively inhibits bacterial growth, highlighting its potential as a natural antibacterial agent. Additionally, antioxidant activity was evaluated using the DPPH assay in 96-well plates, confirming its strong radical-scavenging ability. The study suggests that hibiscus extract could be explored for its synergistic effects when combined with conventional antibiotics.

Keywords: Hibiscus extract; Antibacterial activity; *Salmonella typhi*; *Staphylococcus aureus*; Phytochemicals; Antibacterial susceptibility testing (AST); Minimum inhibitory concentration (MIC); Antioxidant activity

1. Introduction

Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. Natural extracts are frequently in use for developing functional foods and treating illnesses.

The alarming rise of antibiotic-resistant pathogens has necessitated the search for alternative antimicrobial agents. Medicinal plants have been extensively studied for their bioactive compounds that possess therapeutic properties. *Hibiscus rosa-sinensis*, commonly known as China rose, is widely used in traditional medicine to treat infections and inflammation. The antibacterial properties of hibiscus extracts have been attributed to its diverse phytochemicals, including flavonoids, tannins, and saponins.

This study focuses on evaluating the antibacterial potential of hibiscus flower extracts against *S. typhi* and *S. aureus*. *S. aureus* is a common skin flora that can cause serious infections, including wound infections, pneumonia, and sepsis. *S. typhi*, the causative agent of typhoid fever, remains a major public health concern in developing countries. Both pathogens have demonstrated increasing resistance to antibiotics, making them ideal for testing natural antimicrobial agents. By conducting this research, we aim to validate the efficacy of hibiscus extract as a potential antibacterial agent and explore its applications in pharmaceuticals and biotechnology.

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2. Literature Review

Studies have demonstrated that *H. rosa-sinensis* is rich in polyphenols, flavonoids, and organic acids, which contribute to its antimicrobial activity. Research by Al-Mamun et al. (2023) confirmed the antimicrobial properties of *H. rosa-sinensis* extracts, suggesting their potential as natural therapeutic agents. Patel et al. (2018) reported significant antibacterial activity in aqueous extracts of *H. rosa sinensis* leaves against *Aeromonas hydrophila*. However, there is limited research on the antimicrobial potential of its flowers. This study aims to address this gap by evaluating the antibacterial activity of *H. rosa-sinensis* flower extracts using standardized methodologies.

Flavonoids disrupt bacterial cell walls and inhibit nucleic acid synthesis, while tannins interfere with bacterial adhesion and biofilm formation. Alkaloids contribute to antimicrobial action by inhibiting enzymatic activity, whereas saponins function as surfactants that disrupt bacterial membranes. Additionally, the antioxidant properties of hibiscus extracts contribute to their antibacterial efficacy by weakening bacterial defense mechanisms.

3. Methodology

3.1. Preparation of extract of *Hibiscus rosa-sinensis* flowers

The preparation of *Hibiscus rosa-sinensis* flower extract involves several systematic steps to ensure the extraction of bioactive compounds effectively. Fresh flowers of *H. rosa-sinensis* are first collected and thoroughly washed with distilled water to remove any impurities. These flowers are then shade-dried at room temperature for about 5-7 days to preserve their phytochemical constituents. Once completely dried, they are finely powdered using a grinder and stored in an airtight container for further processing.

For the extraction process, a Soxhlet apparatus is used, with methanol as the solvent. Approximately 50 grams of the dried flower powder is placed in the Soxhlet extractor, ensuring proper immersion in the solvent. The extraction is carried out for 48 hours to facilitate maximum extraction of bioactive compounds. Once the extraction is complete, the solvent is removed on a glass dish to dry in the oven at 50°C.

3.2. Antimicrobial Susceptibility Testing (AST) – Kirby-Bauer Method

3.2.1. Preparation of MacFarland Standard

0.5 MacFarland turbidity standard was prepared by mixing 0.5 mL of 1.175% barium chloride solution with 99.5 mL of 1% sulfuric acid. The prepared standard was used to adjust bacterial suspensions to $\sim 1.5 \times 10^8$ CFU/mL for antimicrobial testing.

3.2.2. Antimicrobial Susceptibility Testing (AST) – Kirby-Bauer Method

Mueller-Hinton agar plates were prepared and left to solidify. Bacterial suspensions, standardized to a 0.5 McFarland turbidity, were evenly spread across the agar surface using sterile swabs. Antibiotic discs and hibiscus extract-infused discs were carefully placed on the plates under aseptic conditions. The antibiotic discs used for *Staphylococcus aureus* included Erythromycin (10 µg), Ampicillin (35 µg), Methicillin (5 µg), and Novofloxacin (10 µg). For *Salmonella typhi*, discs included Chloramphenicol (30 µg), Nitrofurantoin (100 µg), Gentamicin (10 µg), and Ofloxacin (5 µg). The plates were then incubated at 37 °C for 24 hours, after which the inhibition zones surrounding the discs were measured and analysed to assess antimicrobial efficacy.

The Kirby-Bauer method works on the principle of antibiotic diffusion through agar. A clear zone around the disc indicates effective bacterial inhibition; the size correlates with susceptibility.

3.3. Minimum Inhibitory Concentration (MIC) Determination

A two-fold serial dilution of *Hibiscus rosa sinensis* extract was prepared in nutrient broth, with concentrations ranging from 1 mg/mL to 0.015625 mg/mL. Separate sets of dilution tubes were inoculated with *S. typhi* and *S. aureus* cultures. The tubes were then incubated at 37 °C for 24 hours, and the lowest concentration that completely inhibited visible bacterial growth was identified as the minimum inhibitory concentration (MIC).

3.4. Phytochemical Screening

Phytochemical screening of *Hibiscus rosa sinensis* extract was performed using qualitative chemical tests to identify key bioactive compounds:

3.4.1. Test for Tannins

Lead Acetate Test: 3 mL of extract was mixed with 3 mL of lead acetate ($\text{Pb}(\text{CH}_3\text{COO})_2$).

The appearance of a white precipitate indicated the presence of tannins.

- Ferric Chloride Test: 3 mL of extract was added to 3 mL of 5% FeCl_3 . The formation of a blue-black coloration indicated the presence of tannins.
- Potassium Dichromate Test: 2 mL of extract was mixed with 2 mL of $\text{K}_2\text{Cr}_2\text{O}_7$ solution. A dark coloration confirmed the presence of tannins.

3.4.2. Test for Flavonoids

- Ferric Chloride Test: 2 mL of extract was mixed with neutral FeCl_3 solution. The formation of a green colour confirmed the presence of flavonoids.
- Shinoda Test: 2 mL of extract was mixed with 1 mL of concentrated HCl and a pinch of magnesium ribbon. The appearance of pink/red/orange coloration indicated the presence of flavonoids.

3.4.3. Test for Triterpenes

- Salkowski's Test: 2 mL of extract was mixed with concentrated H_2SO_4 . A yellow coloration in the lower layer indicated the presence of triterpenes.
- Liebermann's Test: 2 mL of extract was mixed with acetic anhydride and 1 mL of concentrated H_2SO_4 . A deep red coloration at the junction indicated the presence of triterpenes.

3.4.4. Test for Saponins

A. Foam test: 2ml of extract+2ml H_2O - stable froth formation indicated saponins.

3.4.5. Test for Coumarins

A. Fluorescence Test: 2 mL of extract was mixed with 2 mL of 10% NH_4OH . The observation of blue/green fluorescence under UV light confirmed the presence of coumarins.

3.4.6. Test for Quinones

A. Concentrated H_2SO_4 Test: 2 mL of extract was treated with 1 mL of concentrated H_2SO_4 . The formation of a yellow coloration indicated the presence of quinones.

3.4.7. Test for Volatile Oils

A. Staining Test: 1 mL of extract + 1 mL of Sudan III → Persistent oil stain indicated the presence of volatile oils.

3.4.8. Test for Fixed Oils

A. Filter Paper Test: A drop of extract was placed on filter paper and allowed to evaporate → Persistent stain confirmed the presence of fixed oils.

3.5. Antioxidant Activity – DPPH Assay

The antioxidant potential of *Hibiscus rosa sinensis* extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Gallic acid (100 $\mu\text{g}/\text{mL}$) served as the standard. The extract was diluted in methanol at ratios of 1:10, 1:25, and 1:50. A 0.1 mM DPPH solution in methanol was prepared and combined with both the standard and extract dilutions. The mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was recorded at 492 nm using an ELISA plate reader, and the radical scavenging activity was calculated and plotted against concentration.

4. Results

4.1. Extraction of *Hibiscus rosa sinensis*

The methanolic extract of *Hibiscus rosa sinensis* was obtained using aqueous ethanol as a solvent through Soxhlet extraction. This process yielded a dark red, viscous concentrate, characteristic of the plant's natural pigments.

4.2. Antimicrobial Activity of *H. rosa sinensis* Extract

The methanolic extract of *H. rosa sinensis* flowers demonstrated moderate antimicrobial activity. The extract showed greater inhibition against the Gram-positive pathogen *S. aureus*, with a zone of inhibition (ZOI) of 8mm at a 2.5 mg/mL concentration, compared to *S. typhi*, which exhibited a ZOI of 5mm. These results suggest that *S. aureus* is more susceptible to the extract than *S. typhi*. This assay is based on the principle that antioxidants reduce DPPH radicals, resulting in a color change from deep violet to pale yellow. A decrease in absorbance at 517 nm reflects the extract's ability to neutralize free radicals.

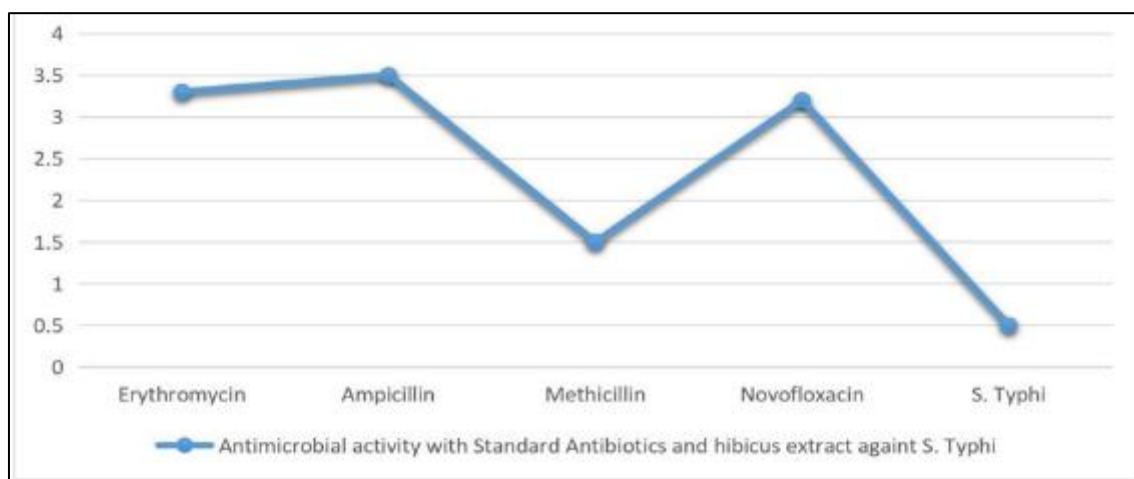


Figure 1 Antimicrobial activity with Standard Antibiotics and Hibiscus extract against *S. typhi*

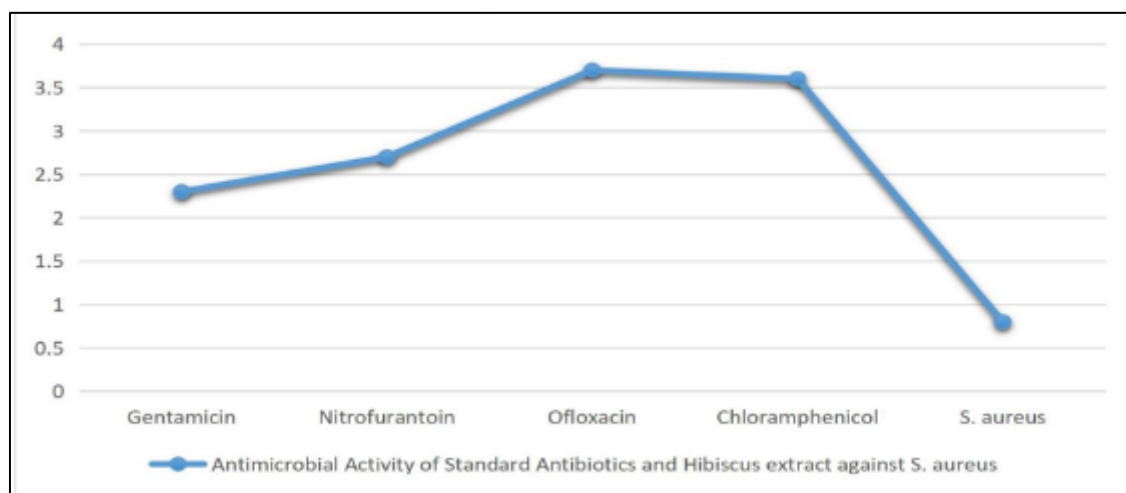


Figure 2 Antimicrobial Activity of Standard Antibiotics and Hibiscus extract against *S. aureus*



Figure 3 Plates depicting the zone sizes against *S. aureus*

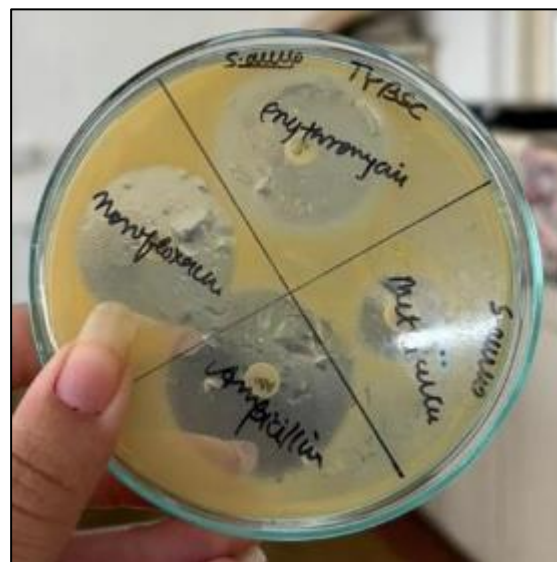


Figure 4 Plates depicting the zone sizes against *S. typhi*

4.3. Minimum Inhibitory Concentration of Aqueous Ethanolic Extract of *Hibiscus rosa sinensis* (Flower)

The minimum inhibitory concentration (MIC) of the crude metabolites from *H. rosa sinensis* flowers demonstrated growth inhibition of *S. aureus* at 0.25 mg/mL, while *S. typhi* exhibited inhibition at 0.5 mg/mL. These findings indicate that *S. aureus* is more susceptible to the extract compared to *S. typhi*.

Table 1 Minimum Inhibitory Concentration of Extract against *S. aureus* and *S. typhi*

Minimum Inhibitory Concentration (MIC) mg/mL		
	<i>S. aureus</i>	<i>S. typhi</i>
MIC value	0.25	0.5



Figure 5 MIC tubes for *S. aureus*



Figure 6 MIC tubes for *S. typhi*

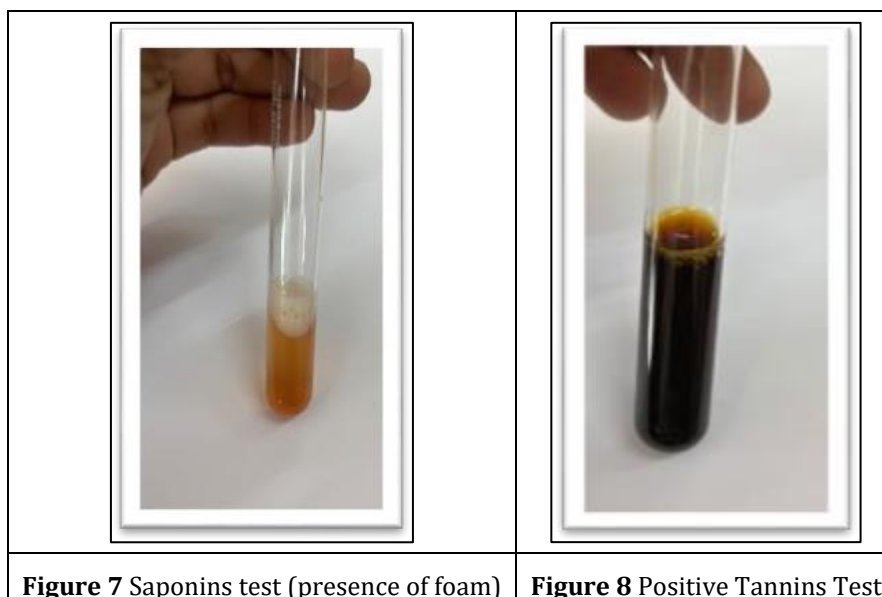
4.4. Phytochemical Screening

The phytochemical analysis of *Hibiscus rosa sinensis* flower extract confirmed the presence of several bioactive compounds, including coumarins, flavonoids, tannins, saponins, and volatile oils.

Table 2 Phytochemical constituents of Aqueous Flower Extract of *Hibiscus rosa sinensis*

	Test	Observation	Inference
1	Tannins Lead Acetate Test Ferric Chloride Test Potassium Dichromate Test:	No reaction No reaction Dark coloration	– – +
2	Flavonoids Ferric Chloride Test Shinoda Test	No pink/red/orange coloration Green colouration	– +
3	Triterpenes Salkowski's Test Liebermann's Test.	No reaction	–
4	Saponins A. Foam test	Stable froth formation	+
5	Coumarins A. Fluorescence Test	Green fluorescence observed under UV	+
6	Quinones A. Concentrated H ₂ SO ₄ Test	No yellow coloration	–
7	Volatile Oils A. Staining Test	Persistent stain observed	+
8	Fixed Oils A. Filter Paper Test	No Persistent stain	–

Key:- (+) = Presence of compound; (–) = Absence of compound

**Figure 7** Saponins test (presence of foam)**Figure 8** Positive Tannins Test

4.5. Antioxidant Activity of *Hibiscus rosa sinensis* Extract

The antioxidant potential of *Hibiscus rosa sinensis* extract was evaluated using the DPPH radical scavenging assay. At a 1:25 dilution, the extract exhibited a radical scavenging activity of 26.8%. The absorbance of the blank (DPPH solution without extract) was recorded at 0.238, while the 1:25 diluted extract showed an absorbance of 0.174. This reduction in absorbance highlights the extract's ability to neutralize free radicals. The observed antioxidant activity is likely due to the presence of phytochemicals such as flavonoids and phenolic compounds, which are well known for their free radical scavenging properties.



Figure 9 Microtitre plate with standard and extract

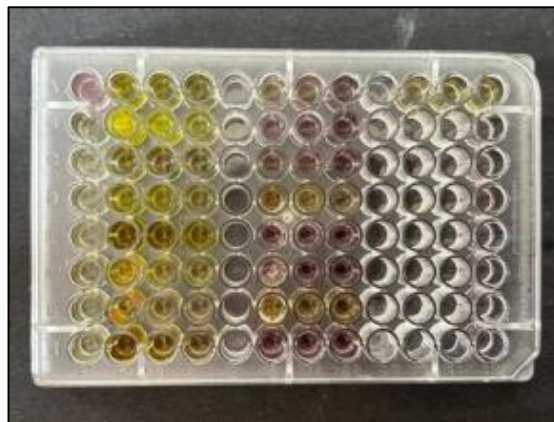


Figure 10 Plate placed in ELISA plate reader



Figure 11 Standard reading at 492nm

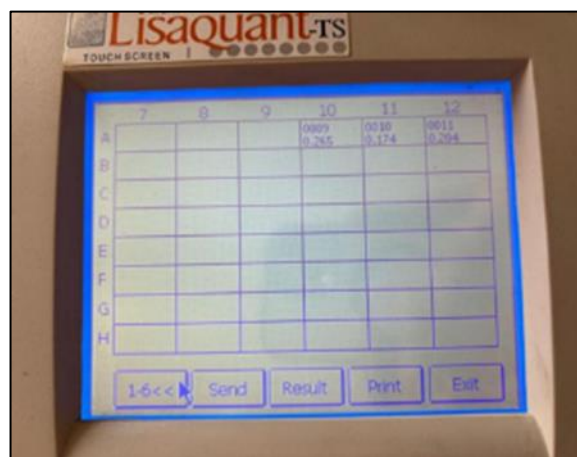


Figure 12 Sample readings at 492nm

5. Discussion

This study investigated the phytochemical composition, antimicrobial efficacy, and antioxidant potential of *Hibiscus rosa-sinensis* flower extract, providing valuable insights into its therapeutic applications. Phytochemical screening confirmed the presence of tannins, flavonoids, coumarins, saponins, and volatile oils in aqueous methanolic extracts. The presence of phytochemicals suggests both physiological and medicinal activities. In contrast, tests for alkaloids, triterpenes, and certain tannins yielded negative results. These findings are consistent with prior literature identifying flavonoids and phenolic compounds as principal bioactive agents in *H. rosa-sinensis*, with flavonoids particularly noted for their ability to inhibit microbial growth and neutralize free radicals [3]. From this general screening it was observed that methanol is a better solvent for extracting phytochemicals.

The antimicrobial assessment, conducted via the Kirby-Bauer disk diffusion and broth dilution methods, revealed notable activity against *Staphylococcus aureus* and *Salmonella typhi*. Among the two, *S. aureus* was more susceptible, requiring lower concentrations of the extract for inhibition. Although the inhibition zones were smaller than those produced by conventional antibiotics, the results suggest a supplementary role for *H. rosa-sinensis* in antimicrobial therapy. The extract's mechanism—likely involving disruption of microbial cell membranes, inhibition of key enzymes,

and interference with protein synthesis—can contribute to its potential as an adjunct to existing antibiotics, possibly reducing the risk of resistance development when used in combination therapies.

In terms of antioxidant capacity, the DPPH assay revealed 26.8% radical scavenging activity at a 1:25 dilution, suggesting moderate free radical neutralization. This activity is attributed to the flavonoids and phenolic compounds, which function by donating hydrogen atoms or electrons to stabilize reactive oxygen species. While the extract demonstrated lower antioxidant capacity compared to gallic acid, the standard reference compound, its activity supports its role as a natural antioxidant source. These results align with previous findings highlighting anthocyanins, flavonoids, and phenolics in *H. rosa-sinensis* as effective agents against oxidative stress [10,11], potentially contributing to the prevention of degenerative and inflammatory conditions.

However, this study is not without limitations. Firstly, the extract was tested in vitro under controlled conditions, which may not fully replicate the complexity of biological systems in vivo. The bioavailability, metabolism, and systemic effects of the active compounds remain unexplored in this context. Secondly, the study focused on a limited number of microbial strains; broader-spectrum testing, including fungal and antibiotic-resistant strains, would provide a more comprehensive antimicrobial profile. Additionally, the specific phytochemicals responsible for the observed bioactivities were not isolated or quantified, which limits the ability to draw precise correlations between compound structure and function. Finally, the antioxidant assay employed only the DPPH method; using multiple antioxidant assays (e.g., ABTS, FRAP) would offer a more robust evaluation of the extract's antioxidant capacity.

In conclusion, while *Hibiscus rosa-sinensis* flower extract demonstrates promising antimicrobial and antioxidant activities—likely due to its flavonoid and phenolic content—further studies are needed to isolate active compounds, evaluate their mechanisms in vivo, and assess their safety and efficacy in clinical settings. Addressing these limitations would pave the way for its potential application in pharmaceutical or nutraceutical formulations.

6. Conclusion

This study comprehensively examined the antimicrobial, phytochemical, and antioxidant properties of *Hibiscus rosa sinensis* extract, highlighting its diverse applications. The Soxhlet extraction process successfully isolated bioactive compounds responsible for its biological effects. Antimicrobial activity was evaluated against *S. aureus* and *S. typhi* using agar well diffusion and MIC determination, while phytochemical screening identified key secondary metabolites. The DPPH assay further provided insights into the extract's antioxidant potential.

The antimicrobial findings revealed greater inhibition against *S. aureus* (0.8 cm ZOI) than *S. typhi* (0.5 cm ZOI), with MIC values of 0.25 mg/mL and 0.5 mg/mL, respectively. These results suggest that Gram-positive bacteria are more vulnerable to the extract, possibly due to structural differences in their cell walls, which facilitate interactions with bioactive compounds. Phytochemical analysis confirmed the presence of flavonoids, tannins, saponins, coumarins, and volatile oils—compounds known for their antimicrobial properties, including bacterial membrane disruption, enzyme inhibition, and interference with metabolic pathways.

The DPPH assay demonstrated the extract's notable antioxidant activity, supporting its role in reducing oxidative stress. As oxidative damage is a key factor in bacterial infections, chronic inflammation, and degenerative diseases, the extract's ability to neutralize reactive oxygen species (ROS) may enhance its antimicrobial effectiveness. These findings suggest that *H. rosa sinensis* extract could serve as a dual-function natural agent with both antimicrobial and antioxidant properties.

As antibiotic resistance and oxidative stress-related diseases continue to pose global health challenges, this study provides a foundation for further research into the therapeutic potential of *H. rosa sinensis*. Future investigations should focus on isolating and characterizing its active compounds, assessing synergistic effects with conventional antibiotics, and conducting in vivo studies and clinical trials to evaluate safety and efficacy. Given its broad-spectrum bioactivity, *H. rosa sinensis* holds promise for applications in pharmaceuticals, food preservation, skincare, and functional nutrition. Harnessing its full potential could lead to innovative natural formulations aimed at combating microbial infections, reducing oxidative stress, and promoting overall health.

7. Future Prospects and Applications

Given the rise of antibiotic resistance, the extract could be explored in combination with conventional antibiotics to enhance their effectiveness against resistant bacteria. It could also be developed into antimicrobial gels, creams, or oral

medications for treating bacterial infections affecting the skin, throat, and digestive system. If proven safe and effective, such herbal treatments could complement modern medicine.

The extract's activity against *S. typhi* suggests potential as a natural preservative in dairy products, fresh produce coatings, or meat packaging. Such plant-based alternatives to synthetic preservatives align well with current consumer demand for clean-label and eco-friendly food products.

The flavonoid rich fraction of petals of *Hibiscus rosa-sinensis* showed effective antioxidant activity. The extract's antioxidant capacity, largely attributed to its polyphenolic content, plays a crucial role in neutralizing free radicals, thereby preventing oxidative damage to the skin. These properties make it highly suitable for inclusion in cosmeceutical products such as anti-aging creams, acne treatments, serums for skin regeneration, and herbal wound dressings. Additionally, its mild astringent nature supports skin toning and pore tightening, which can further enhance its appeal in skincare formulations.

Nanotechnology could enhance the bioavailability and therapeutic efficiency of *H. rosa sinensis* extract. Encapsulating its active compounds in nanoparticles could improve absorption, stability, and targeted delivery. Future studies should investigate its effects on different cancer cell lines to determine its potential as a preventive or therapeutic agent.

In conditions such as typhoid fever, oxidative stress significantly contributes to cellular damage. The high antioxidant capacity of *H. rosa-sinensis* may help alleviate this burden, supporting recovery by reducing inflammation and enhancing immune resilience. While not a standalone treatment, its inclusion in supportive therapy regimens could offer symptomatic relief and faster convalescence.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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